ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

ESMO OPEN SCIENCE FOR OPTIMAL CANCER CARE

ORIGINAL ARTICLE

The genomic landscape of metastatic clear-cell renal cell carcinoma and its prognostic value: a comprehensive analysis of a large real-world clinico-genomic database

M. Rizzo[1*], G. Pezzicoli[2], C. Porta[3], M. Povero[3], L. Pradelli[3], E. Sicari[4], V. S. Barbiero[4] & C. Porta[1,2]

[1]Division of Medical Oncology, AOU Consorziale Policlinico di Bari, Bari; [2]Interdisciplinary Department of Medicine, University of Bari "Aldo Moro", Bari; [3]AdRes HE&OR, Turin; [4]Roche S.p.A., Monza, Italy

Available online xxx

Background: Translating findings on the genomic landscape of metastatic clear-cell renal cell carcinoma (mccRCC) into clinical practice remains challenging. A better understanding of the molecular features of mccRCC could identify a prognostic and/or predictive role for ccRCC genomic alterations.

Patients and methods: In this real-world observational study based on the nationwide (US-based) de-identified Flatiron Health-Foundation Medicine, Inc. clinico-genomic database (FH-FMI-CGDB), we investigate the frequency and co-occurrence of genomic alterations in mccRCC patients and assess their prognostic role. Patients ($n = 858$) were adults diagnosed with mccRCC, with FH electronic health records between 2011 and 2022.

Results: The top 10 mutated genes were *VHL* (73.9%), *PBRM1* (42.4%), *SETD2* (25.3%), *CDKN2A* (20.0%), *BAP1* (16.4%), *CDKN2B* (16.0%), *KDM5C* (14.5%), *TP53* (12.9%), *PTEN* (11.7%), and *TERT* (9.2%). Eight genes showed prognostic value: *CDKN2A*, *CDKN2B*, *TP53*, *PTEN*, *NF2*, *PIK3CA*, and *MTAP* were linked to worse prognosis, whereas *PBRM1* was associated with better overall survival (OS). Two of the three identified gene clusters had prognostic value: cluster 1 (*VHL*, *SETD2*, *PBRM1*, *KDM5C*, *NFE2L2*) correlated with better OS [adjusted hazard ratio (aHR) 0.63, $P < 0.001$], whereas cluster 3 (*CDKN2A*, *CDKN2B*, *BAP1*, *NF2*, *MTAP*) correlated with shorter OS (aHR 1.36, $P = 0.023$).

Conclusion: We identified eight genes and two gene clusters with prognostic significance for mccRCC. Future research will explore the predictive value of gene clusters in various treatments.

Key words: kidney cancer, clear-cell renal cell carcinoma, mutation, real world, clinico-genomic database, prognosis

INTRODUCTION

Renal cell carcinoma (RCC) is the most commonly diagnosed renal neoplasm worldwide,[1] with an estimated 431 288 new cases in 2020.[2] Clear-cell RCC (ccRCC) histotype is the most frequent, accounting for 75% of all RCC cases.[3] Among RCC patients, 33% are diagnosed when the disease is already metastatic. Overall, the 5-year survival rate of RCC patients is 50%; however, despite therapeutic improvements, metastatic patients should still be considered incurable.[4,5]

The therapeutic management of metastatic ccRCC (mccRCC) has undergone many paradigm shifts over the last three decades. Starting from cytokine-based immunotherapy, the systemic treatment of mccRCC was implemented with the introduction of targeted agents, immune checkpoint inhibitors (ICIs), and, more recently, immune-based combinations. This led to a dramatic improvement of median overall survival (OS) from <1 year in the 1990s to >4 years in recent years,[6] but also to new challenges in the management of adverse events.[7]

Currently, the standard of care (SoC) for first-line mccRCC therapy is represented by either a combination of ICI + ICI[7] or of ICI + antiangiogenic agent.[8,9-11] Nevertheless, immuno-combinations fail to elicit deep and durable responses in all patients, and currently no available subsequent second-line treatment is supported by solid scientific evidence.[3,12,13] Moreover, although promising novel treatment options are currently under evaluation, no substantial changes to first-line treatment are expected in the near future.

Genomic profiling of mccRCC could be a step in the right direction to solve the impasse as it could enable the development of more targeted agents, potentially leading to a fully personalized therapeutic approach.[14]

The first whole exome sequencing RCC cohort was published by The Cancer Genome Atlas (TCGA) research

*Correspondence to: Dr Mimma Rizzo, Division of Medical Oncology, University Hospital—Polyclinic of Bari, Piazza G. Cesare, 11, 70124 Bari, Italy. Tel: + 39-0805594508

E-mail: rizzo.mimma@gmail.com (M. Rizzo).

network in 2013[15]; since then, many efforts have been made to investigate the genomic landscape of ccRCC[16,17] and identify genomic alterations that correlate with clinical outcomes.[4,16,18-21]

From these case series, the most common gene alterations in ccRCC were *VHL*, *PBRM1*, *SETD2*, *BAP1*, and *KDM5C*[15-17,22,23] that play a key role in disease development and progression.[24,25]

While inactivation of *VHL*, the most frequently altered gene in sporadic RCC, has no significant prognostic impact,[26] some other genes' alterations influence OS positively (*PBRM1*[27]) or negatively (*SETD2*,[28] *BAP1*,[28] *CDKN2A*,[19] *MTAP*,[19] *PTEN*,[20] and *NF2*[21]). An additional layer of comprehension was added by studying the co-occurrence of these mutations: *PBRM1* is frequently associated with *SETD2*[15] and is mutually exclusive with *BAP1*[15]; *CDKN2A* is frequently associated with *MTAP* and their co-occurrence has been associated with sarcomatoid differentiation and therefore poor prognosis.[19]

Despite advances in understanding mccRCC at the genomic level, translating these findings into common clinical practice remains a challenge because: (i) there is a general lack of genomic profiling case series focusing only on metastatic disease; (ii) many genomic profiling studies were not oriented toward immediate clinical applicability; and (iii) wide genomic profiling panels are difficult to carry out in daily clinical practice.

In this retrospective observational study, we used data from electronic health record (EHR)-derived database to investigate the real-world frequency and co-occurrence of molecular alterations in mccRCC patients and assess their prognostic role.

PATIENTS AND METHODS

Study design and data source

This retrospective observational study relied on the nationwide (US-based) Flatiron Health (FH)-Foundation Medicine, Inc. (FMI) RCC clinico-genomic database (FH-FMI-RCC-CGDB), including data originating from over 280 US cancer clinics (∼800 sites of care, as of January 2024) in the academic and community oncology settings.[29] The data are de-identified and subject to obligations to prevent re-identification and protect patient confidentiality. The CGDB is a patient-level database generated through a deterministic link between the FH longitudinal database sourced from EHR and the FMI genomic database. The de-identified clinical data comprised structured and unstructured data, collected via technology-enabled chart abstraction from physician's notes and other unstructured documents. Genomic data, including specimen features and alteration-level details, were derived from FMI's next-generation sequencing (NGS) tests.[30-32] This analysis was restricted to solid tissue-based assays to ensure a like-to-like comparison between patients profiled using different test types. More details related to data collected and comprehensive genomic profiling (CGP) testing are reported in Supplementary Methods, available at https://doi.org/10.1016/j.esmoop.2025.104294.

Patient population

The RCC-specific cohort within the CGDB was used for this study. The RCC CGDB includes patients who: (i) received a chart-confirmed RCC diagnosis [International Classification of Diseases (ICD)-9 189.x or ICD-10 C64x or C65x]; (ii) had at least two documented clinical visits in the FH network on or after 1 January 2011; and (iii) underwent CGP testing by FMI on a tumor sample collected no earlier than 1 month before the FH diagnosis date.

Selected patients included in this study were adults (≥18 years of age at index date) with a diagnosis of mccRCC between 1 January 2011 and 31 December 2022, who underwent tumor tissue biopsy-based CGP. Patients were followed from their index date, defined as the date of metastatic diagnosis (mDx), until death or the last available observation (within 31 December 2022). Exclusion criteria, applied only to survival outcomes, were: missing mDx; mDx after 30 June 2022 (to ensure a follow-up period of at least 6 months); no visit recorded after the cohort entry date (to exclude patients with no follow-up); and evidence of a clinical study treatment any time before the index date (to remove patients who were previously in clinical trials). Cohort entry date was defined as the date on which a patient enters the study cohort by having met the required inclusion criteria.

Outcomes

The primary co-endpoints were the real-world frequency and co-occurrence of molecular alterations. The mutation rate of each gene was defined as the fraction of the population in which a gene change from the wild-type (WT) to a specific mutant (known or likely pathogenic variant) had been detected at any time. A pair of genes was considered co-mutated if concomitant mutations within single patients were detected.

The secondary endpoint was the prognostic effect of mutated genes on OS, defined as time from the index date until death from any cause or loss to follow-up. The date of death was computed as a composite mortality endpoint, derived from EHR data, obituary records, or Social Security Death Index.[33] Patients without an event were censored on the date of the last available activity.

Statistical analysis

A detailed statistical analysis plan is included in the Supplementary Materials (available at https://doi.org/10.1016/j.esmoop.2025.104294). Baseline characteristics were summarized by using means, standard deviations, medians, and interquartile range (IQR) for continuous variables and counts and percentages for categorical measures. Missing values were treated and reported as a separate category. Alteration frequencies were presented together with exact 95% confidence intervals (CIs) computed using Clopper and Pearson procedure.

A mutual exclusivity/co-occurrence analysis was carried out by using two-sided Fisher's exact test, with Benjamini and Hochberg correction for multiple testing. Odds ratios greater/less than 1 indicate tendencies toward co-occurrence/mutual exclusivity. Clusters of co-occurring

genes were identified via a pre-specified algorithm based on previous published experiences (Supplementary Methods, available at https://doi.org/10.1016/j.esmoop.2025.104294).

OS was analyzed using Kaplan–Meier (KM) methods. As the earliest evidence of CGP may occur after the mDx (i.e. delayed entry), the dataset is left truncated: patients with short survival time who die before undergoing NGS testing are less likely to be observed, introducing a potential bias (i.e. left truncation bias). Risk-set adjustment was used to mitigate the risk of left truncation bias,[34] treating patients at risk only after they have satisfied all inclusion criteria.[35] Entry time was measured from mDx until the later of their first FMI report date, or the second visit date in the FH network, and was set to 0 if negative. The assumption of independent left truncation was verified by fitting a risk-set-adjusted univariate Cox model with entry time as covariate.[36] Univariate and multivariate Cox proportional hazards (PH) models were carried out to assess the prognostic role of each gene (if mutated in at least 20 patients) and cluster. The analyses were run at the gene level to ensure sufficient sample size. The mutational status was defined by the presence of at least one positive test result at each given time point and treated as a time-varying covariate. Patients were considered cluster positive (C+) if they exhibited mutations in at least one gene within the cluster and none in the remaining clusters. This classification did not encompass the *VHL* tumor suppressor gene, as its inactivation has been identified as one of the earliest, pivotal, and most frequent driving events in the development of ccRCC.[15,18,23] The results were presented as hazard ratio (HR) and 95% CI. The following covariates were included as confounders: age, sex, race, Eastern Cooperative Oncology Group (ECOG) performance status (PS) at first line of treatment (L1), and treatment type at L1. The PH assumption was tested with the scaled Schoenfeld residuals. If the assumption was not met, alternative strategies (i.e. stratification for confounders and continuous time-dependent coefficients for exposures) were adopted.

P values <0.05 were considered statistically significant. All the statistical analyses were carried out using R version 4.2.2 (Posit. PBC, Boston, MA).

Sensitivity analyses. Two sensitivity analyses were conducted to assess the robustness of the survival findings. Firstly, we explored the analysis results using the multivariate imputation by chained equations (MICE) method for covariates.[37] MICE imputes missing data iteratively using chained equations, creating multiple datasets (50 in this case) and pooling the results for more robust estimates. In the second analysis, we restricted the cohort to patients who met the inclusion criteria within a time window of 3 months from mDx (i.e. landmark analysis) and applied standard survival analysis methods.

RESULTS

Patient population

The database included 1409 patients with metastatic RCC (mRCC). For 177 (12.6%) the histology was not specified, while 309 patients (21.9%) were diagnosed with non-clear-cell RCC (Supplementary Figure S1, available at https://doi.org/10.1016/j.esmoop.2025.104294). Out of the 923 (65.5%) mccRCC patients, 858 were profiled with tissue-based CGP and were included in this study. Demographic and clinical characteristics of the study cohort are presented in Table 1.

Table 1. Demographic and clinical characteristics of mccRCC patients

Characteristic	Patients (n = 858)
Sex, *n* (%)	
Male	604 (70.4)
Female	254 (29.6)
Age at metastatic diagnosis, years	
Median (IQR)	61 (54-69)
Mean (SD)	61 (11)
Missing, *n*	4
Race, *n* (%)[a]	
White	614 (71.6)
Other race	143 (16.7)
Not reported	49 (5.7)
Black or African American	27 (3.1)
Asian	19 (2.2)
Hispanic or Latino	6 (0.7)
Ancestry, *n* (%)	
EUR	704 (82.1)
AMR	88 (10.3)
AFR	39 (4.5)
EAS/SAS	27 (3.2)
Stage at initial diagnosis, *n* (%)[b]	
I-III	462 (53.8)
IV	380 (44.3)
Not reported	16 (1.9)
Type of diagnosis, *n* (%)[c]	
Recurrent	465 (54.2)
De novo	389 (45.3)
Not reported	4 (0.5)
Systemic treatment, *n* (%)	766 (89.3)
IMCD risk, *n* (%)[d]	
Poor/intermediate risk	521 (68.0)
Favorable risk	66 (8.6)
Unknown	179 (23.4)
Not defined	92
Follow-up time, months[e]	
Median (IQR)	34 (15-60)
Mean (SD)	43 (37)
Missing, *n*	4

AFR, African; AMR, American; EAS, East Asia; EUR, European; IMDC, International Metastatic RCC Database Consortium; IQR, interquartile range; mccRCC, metastatic clear-cell renal cell carcinoma; mRCC, metastatic renal cell carcinoma; SAS, South Asia; SD, standard deviation.

[a]Genomic ancestry for each patient was computed using principal component analysis of single nucleotide polymorphisms trained on data from the 1000 Genomes Project.[32]

[b]Stage was defined by the American Joint Committee (AJC) on Cancer staging system.

[c]*De novo*/recurrent disease was defined as less/more than 90 days between primary diagnosis and diagnosis of metastatic disease.

[d]IMDC risk score was calculated based on the IMDC risk model for metastatic RCC, which predicts survival in patients with metastatic RCC treated with systemic therapy.

[e]Follow-up time was calculated from the date of metastatic diagnosis to the date of death or the date of last follow-up.

A total of 766 (89.3%) patients underwent systemic therapies, with a median number of 2 treatment lines (IQR 1-4, range 1-13) (Supplementary Figure S2, available at https://doi.org/10.1016/j.esmoop.2025.104294). Among them, 521 (68.0%) were classified as poor/intermediate risk according to the International Metastatic Renal Cell Carcinoma Database Consortium (IMDC) criteria and 66 (8.6%) as

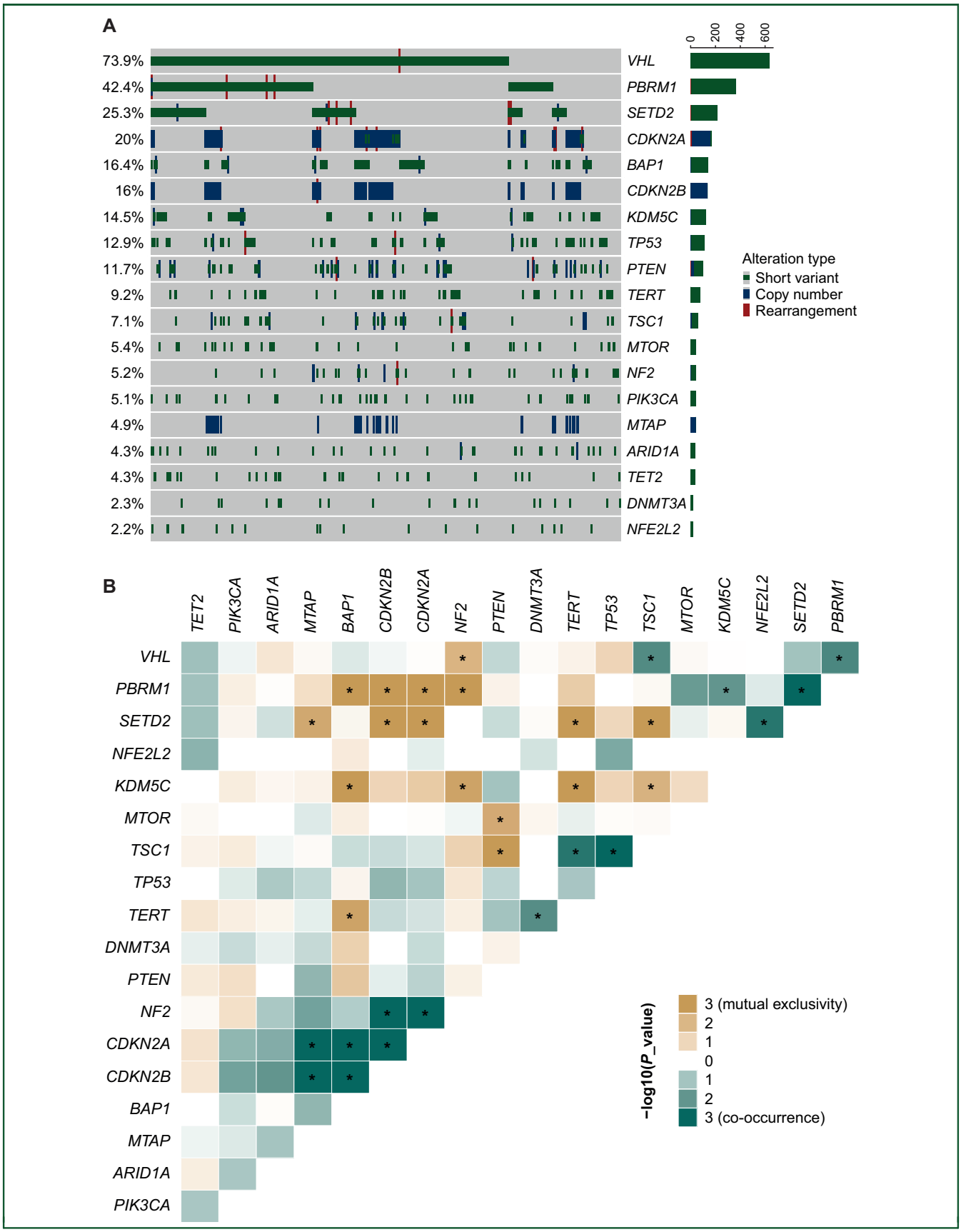

Figure 1. Genomic landscape of ccRCC. (A) Distribution of genomic alterations for the top frequently mutated genes (frequency >2%) across 858 mccRCC samples, color coded by mutation type. Each column corresponds to a single patient. The vertical plot represents the number of mutations in each gene. (B) Co-occurring and mutually exclusive gene pairs. *P* values were calculated using Fisher's exact test. Rows and columns were ordered to highlight cluster patterns. (C) Gene network graph. Nodes represent genes, with their sizes indicating the frequency of alteration and their colors representing the cluster membership. Edge colors indicate the interaction type (green: co-occurrence, gold: mutual exclusivity).

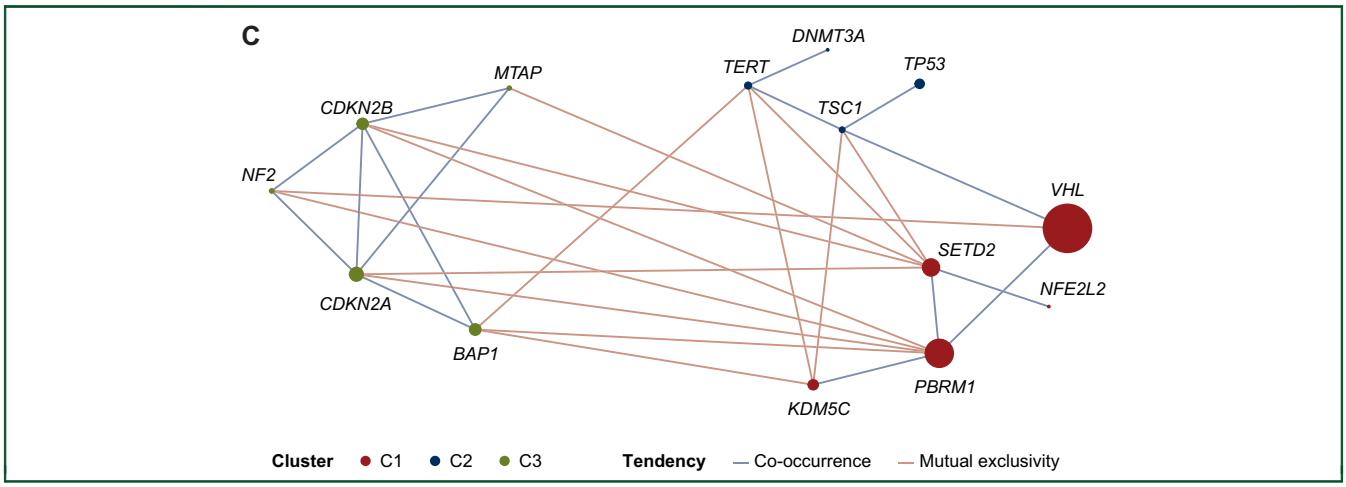

Figure 1. Continued.

favorable risk. The most common L1 therapies were anti-angiogenic (44.8%), followed by immuno-combinations (ICI + ICI 15.1%, ICI + antiangiogenic 13.4%), and experimental treatments (14%) (Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2025.104294). ECOG PS at L1 was available for 453 (59.1%) patients, with 11.7% scoring ≥2 (6.9% of the whole treated cohort).

Specimen collection and testing pattern

A total of 501 (56.7%), 380 (43.0%), and 2 (0.2%) samples were analyzed using FoundationOne® CDx®, FoundationOne®, and FoundationOne®Heme, respectively. In the 883 tissue specimens, 535 (60.6%) were obtained from different metastatic sites (Supplementary Figure S3, available at https://doi.org/10.1016/j.esmoop.2025.104294). Overall, 24 (2.8%) patients underwent multiple profile assessments, with a maximum of three samples collected per patient. Patients profiled with different assays are illustrated in Supplementary Figure S4, available at https://doi.org/10.1016/j.esmoop.2025.104294.

The majority of patients (98.0%) underwent NGS testing after mDx [median time 10.3 months (IQR 1.8-33.3 months)]. Notably, more than half (55.1%) were tested using specimens collected at or before the time of mDx, with a median duration from specimen collection to testing of 3.8 months (IQR 1.2-18.5 months) (Supplementary Table S2, available at https://doi.org/10.1016/j.esmoop.2025.104294).

Genomic landscape of ccRCC

One or more genomic alterations were identified in 99.1% ($n = 850$) of tumors, with a median of 3 mutated genes per patient (IQR 2-5). The genomic landscape of mccRCC is depicted in Figure 1A. The frequency of each mutated gene is reported in Supplementary Table S3, available at https://doi.org/10.1016/j.esmoop.2025.104294. The breakdown of mutation classes for the top mutated genes is shown in Supplementary Figure S5, available at https://doi.org/10.1016/j.esmoop.2025.104294. As expected, *VHL* was the most common altered gene (73.9%), followed by *PBRM1* (42.4%), *SETD2* (25.3%), *CDKN2A* (20.0%), *BAP1* (16.4%), *CDKN2B* (16.0%), *KDM5C* (14.5%), *TP53* (12.9%), *PTEN* (11.7%), and *TERT* (9.2%). The mutual exclusion and co-occurrence of the top mutated genes are displayed in Figure 1B. We identified 32 significant gene interactions (Supplementary Table S4, available at https://doi.org/10.1016/j.esmoop.2025.104294). *CDKN2A* and *CDKN2B* were significantly associated with each other and with *MTAP*, *BAP1*, and *NF2*. *VHL* was enriched with *PBRM1* and *TSC1*. In turn, *PBRM1* showed significant co-occurrence with *SETD2* and *KDM5C*, while *TSC1* co-occurred with *TERT* and *TP53*. *TERT* was enriched with *DNMT3A*. From visual inspection of Figure 1B, three potential clusters of co-occurring genes could be identified: cluster 1—C1 (*VHL*, *SETD2*, *PBRM1*, *KDM5C*, and *NFE2L2*), cluster 2—C2 (*TP53*, *TSC1*, *TERT,* and *DNMT3A*), and cluster 3—C3 (*CDKN2A*, *CDKN2B*, *BAP1*, *NF2*, and *MTAP*). Cluster analysis on the gene network confirmed this hypothesis (Figure 1C). Mutually exclusive patterns were observed between C1 and C3, and C1 and C2 genes. Notably, *BAP1* was mutually exclusive with *PBRM1*. Other mutually exclusive pairs were: *TERT—BAP1*, *PTEN—MTOR*, and *PTEN—TSC1*.

Actionability. Level 1 genomic alterations (OncoKB[38]) for any tumor type were found in 31.9% ($n = 274$) of patients, while only 2.0% ($n = 17$) could be treated with a Food and Drug Administration (FDA)-approved drug. Additionally, based on clinical trials with active enrollment as of December 2023 (https://clinicaltrials.gov/), 48.1% ($n = 413$) and 30.0% ($n = 257$) of patients could be enrolled in a clinical trial in the United States and Europe, respectively. The list of detected actionable alterations is reported in

Edges with zero weight were not depicted. ccRCC, clear-cell renal cell carcinoma.
*Adjusted $P < 0.05$.

Table 2. Prognostic implications according to mutated genes and gene clusters by univariate analysis and multivariate analysis

	n (%)	Median survival, months (95% CI)		Univariate Cox		Multivariate Cox	
		WT	MUT	cHR (95% CI)	*P* value	aHR (95% CI)	*P* value
Mutated genes							
VHL	580 (74.2)	23 (15-32)	27 (21-32)	1.03 (0.83-1.28)	0.8	1.02 (0.82-1.27)	0.8
PBRM1	337 (43.1)	21 (15-29)	32 (26-38)	0.82 (0.68-0.99)	0.04	0.81 (0.67-0.98)	0.033
SETD2[a,b]	199 (25.4)	27 (21-32)	22 (14-34)	0.92 (0.74-1.15)	0.5	1.03 (0.83-1.30)	0.8
CDKN2A	152 (19.4)	29 (24-34)	12 (9.5-20)	1.81 (1.45-2.26)	<0.001	1.91 (1.52-2.41)	<0.001
BAP1	125 (16.0)	26 (20-32)	24 (16-32)	1.14 (0.89-1.47)	0.3	1.13 (0.87-1.47)	0.4
CDKN2B	125 (16.0)	29 (24-34)	11 (8.5-18)	2.02 (1.60-2.55)	<0.001	2.21 (1.73-2.82)	<0.001
KDM5C[a,b]	119 (15.2)	23 (17-29)	40 (32-56)	0.77 (0.58-1.01)	0.059	0.76 (0.57-1.01)	0.055
TP53	98 (12.5)	28 (22-32)	18 (12-26)	1.55 (1.20-2.02)	<0.001	1.48 (1.13-1.94)	0.004
PTEN	88 (11.3)	27 (20-32)	23 (10-31)	1.39 (1.05-1.83)	0.021	1.35 (1.02-1.79)	0.039
TERT	73 (9.3)	24 (18-30)	35 (28-47)	0.9 (0.63-1.28)	0.6	0.89 (0.62-1.27)	0.5
TSC1	57 (7.3)	25 (19-30)	32 (20-55)	0.86 (0.58-1.26)	0.4	0.81 (0.54-1.21)	0.3
MTOR	45 (5.8)	24 (19-30)	35 (25-46)	1.02 (0.68-1.53)	>0.9	0.96 (0.63-1.44)	0.8
NF2	41 (5.2)	27 (22-32)	12 (4.1-29)	1.84 (1.25-2.72)	0.002	1.82 (1.19-2.80)	0.006
PIK3CA	39 (5.0)	27 (22-32)	9.1 (2.6-27)	2.02 (1.36-3.00)	<0.001	2.02 (1.35-3.03)	<0.001
MTAP	36 (4.6)	27 (21-31)	12 (9.3-28)	2.36 (1.59-3.53)	<0.001	2.6 (1.70-3.96)	<0.001
TET2	34 (4.3)	25 (19-30)	30 (22-52)	1.12 (0.71-1.75)	0.6	1.12 (0.70-1.77)	0.6
ARID1A	32 (4.1)	26 (21-31)	13 (6.3-52)	1.33 (0.85-2.09)	0.2	1.5 (0.95-2.38)	0.081
Gene clusters							
C1	291 (37.2)	19 (15-25)	40 (34-49)	0.61 (0.50-0.75)	<0.001	0.63 (0.51-0.78)	<0.001
C2	61 (7.8)	26 (19-31)	28 (17-42)	1.1 (0.78-1.56)	0.6	1.02 (0.71-1.46)	>0.9
C3	104 (13.3)	27 (23-32)	18 (12-29)	1.32 (1.01-1.71)	0.04	1.36 (1.04-1.78)	0.023

aHR, adjusted hazard ratio; C, cluster; cHR, crude hazard ratio; CI, confidence interval; MUT, mutated; PH, proportional hazards; WT, wild type.
[a]Violation of PH assumption in univariate analysis.
[b]Violation of PH assumption in multivariate analysis.

Supplementary Table S5, available at https://doi.org/10.1016/j.esmoop.2025.104294.

Tumor mutational burden and microsatellite instability. Tissue tumor mutational burden (tTMB) was evaluated in 639 samples, with a median value of 2.6 mut/MB (IQR 1.3-5 mut/MB). In the tTMB-assessable population, only 2.4% of patients ($n = 15/623$) had high tTMB (>10 mut/MB). Out of the 735 samples examined for microsatellite instability (MSI), 88.4% ($n = 650$), 0.7% ($n = 5$), and 10.9% ($n = 80$) harbored microsatellite stable, MSI-intermediate (MSI-I), and MSI unknown, respectively; none showed MSI-high (MSI-H).

Survival

Overall, 782 (91.1%) patients were assessable for survival outcomes (the baseline characteristics are reported in Supplementary Table S6, available at https://doi.org/10.1016/j.esmoop.2025.104294). Median OS was 26 months (95% CI 20-30 months), with a 60-month survival rate of 19% (95% CI 15% to 22%). KM plots of top mutated genes are presented in Supplementary Figure S6, available at https://doi.org/10.1016/j.esmoop.2025.104294. Univariate and multivariate Cox PH analysis showed that eight genes were significantly related to OS (Table 2). In the phosphoinositide 3-kinase/protein kinase B/mammalian target of rapamycin signaling pathway, *PIK3CA* [adjusted HR (aHR) 2.02, $P < 0.001$] and *PTEN* (aHR 1.35, $P = 0.039$) had a negative impact on prognosis. Patients with *CDKN2A*/*CDKN2B* mutations were correlated with decreased survival compared with *CDKN2A*/*CDKN2B* WT patients (aHR 1.91 and 2.21, respectively, $P < 0.001$). Similar associations were found for tumor suppressors *TP53* (aHR 1.48, $P = 0.004$), *NF2* (aHR 1.82, $P = 0.006$), and *MTAP* (aHR 2.60, $P < 0.001$). Mutant *PBRM1* had better OS (aHR 0.81, $P = 0.033$), while no correlation was found between survival outcomes and the other chromatin remodeling modulator genes *BAP1* and *SETD2*. *VHL* had no prognostic value in this cohort. *KDM5C* was associated with a decreased risk of death early on ($HR_{t = 0}$ 0.46), and then its effect tended to vanish ($HR_{t = 60\ months}$ 1.01) (Supplementary Figure S7, available at https://doi.org/10.1016/j.esmoop.2025.104294).

Prognostic role of gene clusters. A total of 291 (37.2%), 61 (7.8%), and 104 (13.3%) patients were categorized as C1+, C2+, and C3+, respectively (Table 2). KM curves for the three clusters are displayed in Figure 2 and Supplementary Figure S6, available at https://doi.org/10.1016/j.esmoop.2025.104294. C1+ patients had longer OS compared with C1− (aHR 0.63, $P < 0.001$), while positivity to C3 predicted shorter OS (aHR 1.36, $P = 0.023$). No association was observed between C2 and OS (aHR 1.02, $P > 0.9$). Among treated cases, C1+ patients were more frequently classified as favorable risk compared with C1− (11.5% C1+ versus 6.7% C1−, $P = 0.001$). No significant differences between C2+ and C2−, and C3+ and C3− patients were observed in IMDC distribution.

Sensitivity analyses. Sensitivity analyses using MICE and landmark analyses yielded similar results to the base case (Supplementary Tables S7 and S8, available at https://doi.org/10.1016/j.esmoop.2025.104294).

DISCUSSION

In this study, we analyzed the results of CGP carried out on tissue samples in a real-world population of mccRCC patients included in a clinico-genomic database. To the best of

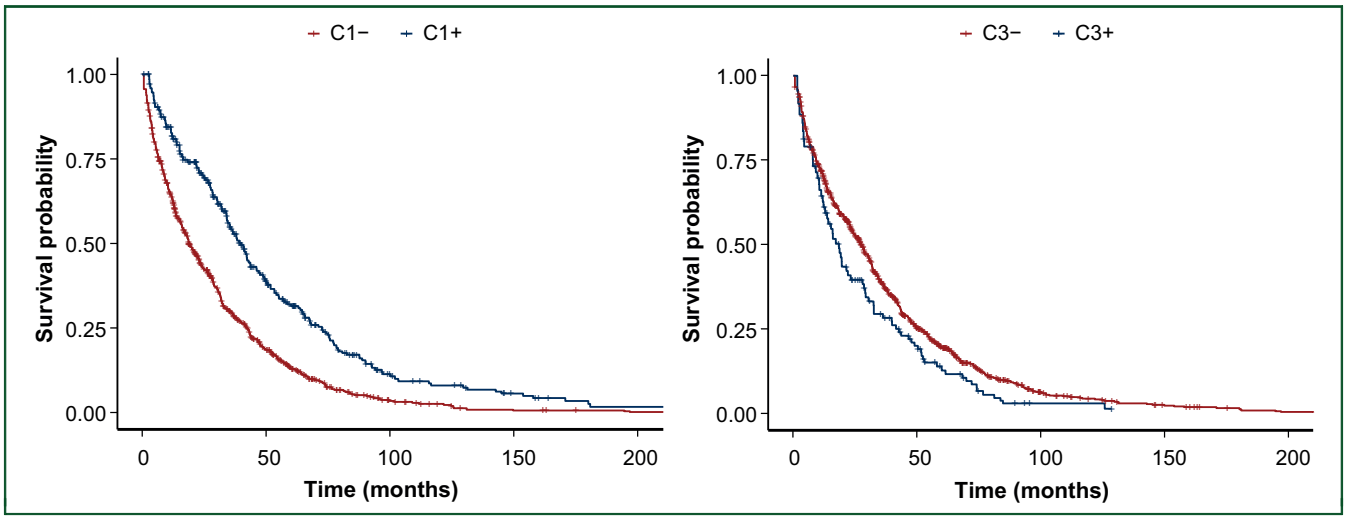

Figure 2. Kaplan–Meier curves of C1 and C3. Plotted *P* values were derived from the univariate Cox proportional hazards regression model.
C, cluster.

our knowledge, this is the largest analysis linking the clinical and genomic landscape of mccRCC, with 858 patients investigated.

The demographic and clinical features of our study population were comparable to those of contemporary real-world mRCC case series[2,39,40] encompassing mainly white, male patients of European origin. Given the enrollment time, and the average length of follow-up, the distribution of L1 therapies was consistent with recently published retrospective mccRCC cohorts.[41,42] In our series, the most represented first-line treatments were antiangiogenic monotherapies (44.8%), which have been the first-line SoC[43-46] over more than a decade, and immuno-combinations (28.5%), which have demonstrated clinical benefit over antiangiogenic monotherapy[47-51] and represent the newest first-line SoC.

The genomic landscape reported in our study was consistent with that previously described by TCGA research network[15,16] and the major genomic profiling cohorts.[17,22,23,52,53] *VHL* gene mutation was the most frequent alteration (73.9% of tumors), followed by mutations in chromatin remodeling modulator genes, such as *PBRM1* (42.4%), *SETD2* (25.3%), and *BAP1* (16.4%).

The analysis of the prognostic effect of mutated genes on OS confirmed the known positive prognostic value of *PBRM1*[27] and negative prognostic value of *CDKN2A*,[19] *MTAP*,[19] *PTEN*,[20] *PIK3CA*,[54] and *NF2*.[20] Conversely, *SETD2* and *BAP1*, which showed a prognostic role in smaller populations,[18] showed no impact on survival. Our findings also confirmed the positive prognostic role of *KDM5C*.[55] However, this gene violated the PH assumption in multivariate analyses and the prognostic effect on OS varied over time. Further analyses are needed to better understand the mechanisms underlying its prognostic role.

2.4% of our cohort had high TMB (≥10 mut/MB), 0.8% ($n = 5/655$) had MSI-I, and 0% had MSI-H. This is in line with the literature data,[56,57] which highlight the scarce tendency of ccRCC toward high TMB or high mutation susceptibility, such as in the case of MSI-H tumors. Interestingly, patients with high TMB showed worse survival (aHR 2.09, $P = 0.027$), in contrast to other studies published in the literature, such as that of Samstein et al.[58] who, among RCC patients treated with ICI, described a better OS in subjects with high TMB versus low TMB. It should be noted that the study by Samstein et al. differs from our study in the fist-line treatment and in the TMB-high definition.[58] Therefore, the potential role of TMB as a predictor of response to ICI should be explored in future dissertations.

Our results of the co-occurrence alterations are in line with those reported by Clark et al.[23] and showed that *VHL* mutations were co-occurring with other early alterations in *PBRM1*, a 3p chromosome gene.[59] In addition, we observed that *PBRM1* mutations showed significant co-occurrence with *SETD2* and *KDM5C* mutations, but they were mutually exclusive with *BAP1* mutations. The mutual exclusion between *PBRM1* and *BAP1* was well established in the case series by Bihr et al.,[60] and their role as lineage-specific drivers was elucidated in the *in vivo* study by Gu et al.[61] We also found a significant association between *CDKN2A* and *MTAP*, whose co-occurrence was associated with sarcomatoid differentiation in a case series by Xu et al.[19]

Mutations of *PBRM1*, *SETD2*, and *KDM5C* were among the most common co-occurring gene mutations in ccRCC, following *VHL*, and they were included in C1. The positive prognostic impact of genes included in C1 observed in our study (aHR 0.63) was also reported by Liu et al.[62] who highlighted that ccRCC with *PBRM1* mutations was usually associated with a favorable prognosis, even if the co-occurrence of *SETD2* mutations, with or without *KDM5C* mutations, could reduce this protective effect.

C2 encompassed *TERT* (the telomerase reverse transcriptase), *DNMT3A* (a gene coding for a protein involved in DNA methylation), and two tumor suppressors, *TP53* and *TSC1*. The association between these genes was only recently described in a ccRCC cohort by Mar et al.,[63] who showed frequent co-mutations of *TP53* and *TSC1* in *TERT*-mutated ccRCC. In our cohort, no association was found

between C2 and OS (aHR 1.02). To the best of our knowledge, this is the first study that evaluated the prognostic impact of these genes in ccRCC. Future analyses could further investigate the prognostic effect of this cluster.

In C3, we included *CDKN2A/B* (cell cycle-regulating kinases), *BAP1* (a chromatin modulator), *NF2* (a tumor suppressor), and *MTAP* (an enzyme implied in polyamine metabolism). C3+ patients showed worse survival than C3− patients (aHR 1.36). Interestingly, the association between *NF2*, *CDKN2A/B*, and *BAP1* has already been described in the literature and showed a correlation with sarcomatoid features and programmed death-ligand 1 expression.[21] Moreover, the co-occurrence of *CDKN2A* and *MTAP* was associated with sarcomatoid features and a worse prognosis.[19,64,65]

This study suggests that data on ccRCC genomic alterations obtained using a standardized commercially available test could be integrated into clinical practice. Firstly, genomic profiling could identify specific biomarkers that can be treated with FDA-approved treatments. However, only 2.0% of the patients in our series could have received FDA-approved treatments because of the specific alterations identified in their tumors. The test also identified alterations that could have allowed the enrollment of patients in specific clinical trials. Approximately, 30.0% and 48.1% of the patients included in the present analysis could have been enrolled in biomarker-driven clinical trials in Europe and the United States, respectively. This is of utmost importance if we consider that among the major basket trials of the last decade (i.e. HERALD,[66] MOSCATO,[67] SHIVA,[68] WINTHER,[69] ProfilER,[70] myPathway,[71] MATCH,[72] I-PREDICT,[73] and DRUP[74]) RCC patients were often not included or were present in a dismal percentage (1%-4.2%). In our study, consistent with other real-world data, most patients (86.5%) were profiled after evidence of metastases with a median time from mDx to NGS testing of 14.5 months. Vasudev et al.[75] correlated the disease-free survival of patients with radically resected RCC with their genomic alterations, and they developed an algorithm to select patients who should receive adjuvant treatment based on the number of genomic alterations.[75] In the light of the latest therapeutic advances, genomic profiling should also be considered in patients with localised ccRCC, so as not to preclude them from accessing adjuvant SoC and biomarker-driven clinical trials, and should be repeated at metastatic disease progression.

Furthermore, knowing the genetic clusters of each tumor could help mccRCC decision making in clinical practice. C1+ patients showed a favorable prognosis and a longer OS from mDx; therefore, they tend to have a better duration of response to systemic therapies used in clinical practice. C1+ patients obtained good treatment outcomes both with sequential monotherapies and with immuno-combinations; therefore, these patients should be carefully evaluated since the option of therapeutic de-escalation (sequential monotherapies instead of immuno-combinations) should be considered. Conversely, C3+ patients showed a worse prognosis and less response to common clinical practice therapies. These patients should be considered for clinical trial enrollment, particularly in biomarker-driven basket trials.

Lastly, knowing the genomic landscape of patients with mRCC should be considered as an investment, whose value can only grow over time: mutations that today are considered undruggable could become druggable in the foreseeable future. This has happened recently with the introduction of *KRAS* inhibitors,[76] and it is currently happening with the first positive clinical results of p53 structural correctors.[77] The latter class of molecules might have some relevance in mccRCC management, since *TP53* mutations are quite common in this population (12.9% of the patients in our series).

This research is subject to several limitations, some of which are inherent in most observational studies, such as unmeasured confounding, misclassification, and incomplete data entry. The cohort comprised US patients treated primarily at community centers who underwent CGP testing as a part of their care. Due to this selection bias, the analysis involves a type of patient who may not be representative of the entire population of mccRCC patients, and results may not be generalizable to a broader patient population. Nonetheless, the information obtained from the database is reflective of the real-world patient population.

The majority of patients in the database have only a single biopsy available for testing, which limits our ability to fully characterise the mutational landscape of ccRCC, given the well-documented intratumoral heterogeneity.[78] This limitation is less evident in biopsies from metastatic sites, which are more prone to represent the dominant subclone driving metastasis. However, single-region biopsies from primary tumors may not adequately capture the molecular profile of the metastasizing clone due to the high intratumoral heterogeneity.[78-80] Furthermore, the use of a single-region biopsy methodology may result in an underestimation of mutational co-occurrence, which could potentially compromise the accuracy of the clustering analysis. In order to corroborate the prognostic relevance and composition of the identified clusters, an external validation is required.

Other biases are related to the time of enrollment, as patients enrolled earlier had a longer follow-up than patients enrolled later, which may have biased the survival analysis, and the change in the therapeutic landscape during the 12-year observation period, which could make the observed treatment pattern not representative of current clinical practice.

Lastly, the CGDB is subject to left truncation: in order for patients to be included in the database, they must first have survived long enough to receive NGS testing. To account for left truncation bias, we applied risk-set adjustment, which relies on the assumption of independence between survival and entry time. This assumption was nearly met (HR 1.02, 95% CI 1.02-1.03), indicating a modest degree of dependent left truncation. As a consequence, survival estimates

obtained using risk-set adjustment might be biased. However, the bias was likely to be low, because landmark analysis results were generally consistent with the primary findings, despite being conducted on a limited sample size. Overall, sensitivity analyses suggested robustness in our conclusions.

In conclusion, we reported the genomic landscape of a large cohort of patients with mccRCC and identified two prognostic clusters of co-occurring genes. These findings contribute to a better understanding of the molecular features of mccRCC and promote further analyses focused on validating the predictive role of ccRCC genomic alterations and finding the biological basis for gene clusters to increase recruitment into biomarker-driven clinical trials, aid clinicians in therapeutic choice, and improve personalized therapeutic strategies.

ACKNOWLEDGEMENTS

Writing assistance and journal styling services were provided by SEEd Medical Publishers.

FUNDING

This project was sponsored and financially supported by Roche S.p.A. (no grant number).

DISCLOSURE

ES and VSB are employed by Roche S.p.A. CP and MP are employees of AdRes s.r.l, which has received project funding from Roche for the conduct of the study. LP is a co-owner and employee of AdRes, which has received project funding from Roche for the conduct of the study. All other authors have declared no conflicts of interest.

REFERENCES

1. The Surveillance, Epidemiology, and End Results (SEER) Program. Cancer Statistics. SEER. Available at https://seer.cancer.gov/statistics/index.html. Accessed March 18, 2024.
2. Bukavina L, Bensalah K, Bray F, et al. Epidemiology of renal cell carcinoma: 2022 update. *Eur Urol*. 2022;82(5):529-542.
3. Chevrier S, Levine JH, Zanotelli VRT, et al. An immune atlas of clear cell renal cell carcinoma. *Cell*. 2017;169(4):736-749.e18.
4. Tennenbaum DM, Manley BJ, Zabor E, et al. Genomic alterations as predictors of survival among patients within a combined cohort with clear cell renal cell carcinoma undergoing cytoreductive nephrectomy. *Urol Oncol*. 2017;35(8). 532.e7-532.e13.
5. Scholtes MP, Alberts AR, Iflé IG, Verhagen PCMS, van der Veldt AAM, Zuiverloon TCM. Biomarker-oriented therapy in bladder and renal cancer. *Int J Mol Sci*. 2021;22(6):2832.
6. Demasure S, Spriet I, Debruyne PR, et al. Overall survival improvement in patients with metastatic clear-cell renal cell carcinoma between 2000 and 2020: a retrospective cohort study. *Acta Oncol*. 2022;61(1):22-29.
7. Santoni M, Rizzo M, Burattini L, et al. Present and future of tyrosine kinase inhibitors in renal cell carcinoma: analysis of hematologic toxicity. *Recent Pat Antiinfect Drug Discov*. 2012;7(2):104-110.
8. Tannir NM, Escudier B, McDermott DF, et al. Nivolumab plus ipilimumab (NIVO+IPI) vssunitinib (SUN) for first-line treatment of advanced renal cell carcinoma (aRCC): long-term follow-up data from the phase 3 CheckMate 214 trial. *J Clin Oncol*. 2024;42(suppl 4):363.
9. Plimack ER, Powles T, Stus V, et al. Pembrolizumab plus axitinib versus sunitinib as first-line treatment of advanced renal cell carcinoma: 43-month follow-up of the phase 3 KEYNOTE-426 study. *Eur Urol*. 2023;84(5):449-454.
10. Bourlon MT, Escudier B, Burotto M, et al. Nivolumab plus cabozantinib (N+C) vs sunitinib (S) for previously untreated advanced renal cell carcinoma (aRCC): results from 55-month follow-up of the CheckMate 9ER trial. *J Clin Oncol*. 2024;42(suppl 4):362.
11. Motzer RJ, Porta C, Eto M, et al. Lenvatinib plus pembrolizumab versus sunitinib in first-line treatment of advanced renal cell carcinoma: final prespecified overall survival analysis of CLEAR, a phase III study. *J Clin Oncol*. 2024;42:1222-1228.
12. Motzer RJ, Jonasch E, Agarwal N, et al. NCCN Guidelines® insights: kidney cancer, version 2.2024. *J Natl Compr Cancer Netw*. 2024;22(1):4-16.
13. Powles T, Albiges L, Bex A, et al. Renal cell carcinoma: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. *Ann Oncol*. 2024;35(8):692-706.
14. Pezzicoli G, Ciciriello F, Musci V, Salonne F, Ragno A, Rizzo M. Genomic profiling and molecular characterization of clear cell renal cell carcinoma. *Curr Oncol*. 2023;30(10):9276-9290.
15. The Cancer Genome Atlas Research Network. Comprehensive molecular characterization of clear cell renal cell carcinoma. *Nature*. 2013;499(7456):43-49.
16. Ricketts CJ, De Cubas AA, Fan H, et al. The Cancer Genome Atlas comprehensive molecular characterization of renal cell carcinoma. *Cell Rep*. 2018;23(1):313-326.e5.
17. Huang J, Cai W, Cai B, et al. Comprehensive genomic landscape in Chinese clear cell renal cell carcinoma patients. *Front Oncol*. 2021;11:697219.
18. Gossage L, Murtaza M, Slatter AF, et al. Clinical and pathological impact of *VHL, PBRM1, BAP1, SETD2, KDM6A*, and *JARID1c* in clear cell renal cell carcinoma. *Genes Chromosomes Cancer*. 2014;53(1):38-51.
19. Xu W, Anwaier A, Liu W, et al. Genomic alteration of MTAP/CDKN2A predicts sarcomatoid differentiation and poor prognosis and modulates response to immune checkpoint blockade in renal cell carcinoma. *Front Immunol*. 2022;13:953721.
20. Lee HJ, Lee HY, Lee JH, et al. Prognostic significance of biallelic loss of *PTEN* in clear cell renal cell carcinoma. *J Urol*. 2014;192(3):940-946.
21. Hacking SM, Pavlick D, Wang Y, et al. Comprehensive genomic profiling of NF2-mutated kidney tumors reveals potential targets for therapy. *Oncologist*. 2023;28(7):e508-e519.
22. Sato Y, Yoshizato T, Shiraishi Y, et al. Integrated molecular analysis of clear-cell renal cell carcinoma. *Nat Genet*. 2013;45(8):860-867.
23. Clark DJ, Dhanasekaran SM, Petralia F, et al. Integrated proteogenomic characterization of clear cell renal cell carcinoma. *Cell*. 2019;179(4):964-983.e31.
24. Davis CF, Ricketts CJ, Wang M, et al. The somatic genomic landscape of chromophobe renal cell carcinoma. *Cancer Cell*. 2014;26(3):319-330.
25. Qiu P. Embracing the dropouts in single-cell RNA-seq analysis. *Nat Commun*. 2020;11(1):1169.
26. Kim BJ, Kim JH, Kim HS, Zang DY. Prognostic and predictive value of VHL gene alteration in renal cell carcinoma: a meta-analysis and review. *Oncotarget*. 2017;8(8):13979-13985.
27. van de Pol JAA, Ferronika P, Westers H, et al. Evaluation of a seven gene mutational profile as a prognostic factor in a population-based study of clear cell renal cell carcinoma. *Sci Rep*. 2022;12(1):6478.
28. Hakimi AA, Ostrovnaya I, Reva B, et al. Adverse outcomes in clear cell renal cell carcinoma with mutations of 3p21 epigenetic regulators BAP1 and SETD2: a report by MSKCC and the KIRC TCGA research network. *Clin Cancer Res*. 2013;19(12):3259-3267.
29. Singal G, Miller PG, Agarwala V, et al. Association of patient characteristics and tumor genomics with clinical outcomes among patients with non—small cell lung cancer using a clinicogenomic database. *JAMA*. 2019;321(14):1391-1399.
30. Frampton GM, Fichtenholtz A, Otto GA, et al. Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. *Nat Biotechnol*. 2013;31(11):1023-1031.

31. Milbury CA, Creeden J, Yip WK, et al. Clinical and analytical validation of FoundationOne®CDx, a comprehensive genomic profiling assay for solid tumors. *PLoS One*. 2022;17(3):e0264138.
32. He J, Abdel-Wahab O, Nahas MK, et al. Integrated genomic DNA/RNA profiling of hematologic malignancies in the clinical setting. *Blood*. 2016;127(24):3004-3014.
33. Carrot-Zhang J, Chambwe N, Damrauer JS, et al. Comprehensive analysis of genetic ancestry and its molecular correlates in cancer. *Cancer Cell*. 2020;37(5):639-654.e6.
34. Keiding N, Moeschberger M. Independent delayed entry. In: Klein JP, Goel PK, editors. *Survival Analysis: State of the Art. Nato Science*. The Netherlands: Springer; 1992. p. 309-326.
35. Agarwala V, Khozin S, Singal G, et al. Real-world evidence in support of precision medicine: clinico-genomic cancer data as a case study. *Health Aff*. 2018;37(5):765-772.
36. Mackenzie T. Survival curve estimation with dependent left truncated data using Cox's model. *Int J Biostat*. 2012;8(1).
37. van Buuren S, Groothuis-Oudshoorn K. mice: Multivariate imputation by chained equations in R. *J Stat Softw*. 2011;45(3):1-67.
38. Chakravarty D, Gao J, Phillips S, et al. OncoKB: a precision oncology knowledge base. *JCO Precis Oncol*. 2017;(1):1-16.
39. Padala SA, Barsouk A, Thandra KC, et al. Epidemiology of renal cell carcinoma. *World J Oncol*. 2020;11(3):79-87.
40. Saad AM, Gad MM, Al-Husseini MJ, Ruhban IA, Sonbol MB, Ho TH. Trends in renal-cell carcinoma incidence and mortality in the United States in the last 2 decades: a SEER-based study. *Clin Genitourin Cancer*. 2019;17(1):46-57.e5.
41. Graham J, Shah AY, Wells JC, et al. Outcomes of patients with metastatic renal cell carcinoma treated with targeted therapy after immuno-oncology checkpoint inhibitors. *Eur Urol Oncol*. 2021;4(1):102-111.
42. George S, Faccone J, Huo S, et al. Real-world treatment patterns and sequencing for metastatic renal cell carcinoma (mRCC): results from the Flatiron database. *J Clin Oncol*. 2021;39(suppl 6):286.
43. Motzer RJ, Hutson TE, Tomczak P, et al. Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. *N Engl J Med*. 2007;356(2):115-124.
44. Sternberg CN, Davis ID, Mardiak J, et al. Pazopanib in locally advanced or metastatic renal cell carcinoma: results of a randomized phase III trial. *J Clin Oncol*. 2023;41(11):1957-1964.
45. Hudes G, Carducci M, Tomczak P, et al. Temsirolimus, interferon alfa, or both for advanced renal-cell carcinoma. *N Engl J Med*. 2007;356(22):2271-2281.
46. Escudier B, Pluzanska A, Koralewski P, et al. Bevacizumab plus interferon alfa-2a for treatment of metastatic renal cell carcinoma: a randomised, double-blind phase III trial. *Lancet*. 2007;370(9605):2103-2111.
47. Motzer RJ, Tannir NM, McDermott DF, et al. Nivolumab plus ipilimumab versus sunitinib in advanced renal-cell carcinoma. *N Engl J Med*. 2018;378(14):1277-1290.
48. Rini BI, Plimack ER, Stus V, et al. Pembrolizumab plus axitinib versus sunitinib for advanced renal-cell carcinoma. *N Engl J Med*. 2019;380(12):1116-1127.
49. Rini BI, Powles T, Atkins MB, et al. Atezolizumab plus bevacizumab versus sunitinib in patients with previously untreated metastatic renal cell carcinoma (IMmotion151): a multicentre, open-label, phase 3, randomised controlled trial. *Lancet*. 2019;393(10189):2404-2415.
50. Motzer RJ, Penkov K, Haanen J, et al. Avelumab plus axitinib versus sunitinib for advanced renal-cell carcinoma. *N Engl J Med*. 2019;380(12):1103-1115.
51. Choueiri TK, Powles T, Burotto M, et al. Nivolumab plus cabozantinib versus sunitinib for advanced renal-cell carcinoma. *N Engl J Med*. 2021;384(9):829-841.
52. Hsieh JJ, Chen D, Wang PI, et al. Genomic biomarkers of a randomized trial comparing first-line everolimus and sunitinib in patients with metastatic renal cell carcinoma. *Eur Urol*. 2017;71(3):405-414.
53. Forbes SA, Bindal N, Bamford S, et al. COSMIC: mining complete cancer genomes in the Catalogue of Somatic Mutations in Cancer. *Nucleic Acids Res*. 2011;39(suppl 1):D945-D950.
54. Li Y, Wang C, Gao Y, Zhou L. Identification and validation of PIK3CA as a marker associated with prognosis and immune infiltration in renal clear cell carcinoma. *J Oncol*. 2021;2021:e3632576.
55. Bossé D, Xie W, Ged Y, et al. Alterations in key clear cell renal cell carcinoma (RCC) genes to refine patient prognosis. *J Clin Oncol*. 2018;36(suppl 15):4516.
56. Alexandrov LB, Nik-Zainal S, Wedge DC, et al. Signatures of mutational processes in human cancer. *Nature*. 2013;500(7463):415-421.
57. Bonneville R, Krook MA, Kautto EA, et al. Landscape of microsatellite instability across 39 cancer types. *JCO Precis Oncol*. 2017;(1):1-15.
58. Samstein RM, Lee CH, Shoushtari AN, et al. Tumor mutational load predicts survival after immunotherapy across multiple cancer types. *Nat Genet*. 2019;51(2):202-206.
59. Walton J, Lawson K, Prinos P, Finelli A, Arrowsmith C, Ailles L. PBRM1, SETD2 and BAP1 - the trinity of 3p in clear cell renal cell carcinoma. *Nat Rev Urol*. 2023;20(2):96-115.
60. Bihr S, Ohashi R, Moore AL, et al. Expression and mutation patterns of PBRM1, BAP1 and SETD2 mirror specific evolutionary subtypes in clear cell renal cell carcinoma. *Neoplasia*. 2019;21(2):247-256.
61. Gu YF, Cohn S, Christie A, et al. Modeling renal cell carcinoma in mice: *Bap1* and *Pbrm1* inactivation drive tumor grade. *Cancer Discov*. 2017;7(8):900-917.
62. Liu Y, Li Y, Xu H, Zhou L, Yang X, Wang C. Exploration of morphological features of clear cell renal cell carcinoma with *PBRM1, SETD2, BAP1*, or *KDM5C* mutations. *Int J Surg Pathol*. 2023;31(8):1485-1494.
63. Mar N, Jaeger EB, Ashok A, Rezazadeh A. Molecular characteristics of advanced clear cell renal cell carcinoma (ccRCC) harboring TERT mutations. *J Clin Oncol*. 2023;41(suppl 6):706.
64. La Rochelle J, Klatte T, Dastane A, et al. Chromosome 9p deletions identify an aggressive phenotype of clear cell renal cell carcinoma. *Cancer*. 2010;116(20):4696-4702.
65. Beroukhim R, Brunet JP, Di Napoli A, et al. Patterns of gene expression and copy-number alterations in von-hippel lindau disease-associated and sporadic clear cell carcinoma of the kidney. *Cancer Res*. 2009;69(11):4674-4681.
66. Taniguchi H, Yagisawa M, Satoh T, et al. Tissue-agnostic efficacy of trastuzumab deruxtecan (T-DXd) in advanced solid tumors with HER2 amplification identified by plasma cell-free DNA (cfDNA) testing: results from a phase 2 basket trial (HERALD/EPOC1806). *J Clin Oncol*. 2023;41:3014.
67. Massard C, Michiels S, Ferté C, et al. High-throughput genomics and clinical outcome in hard-to-treat advanced cancers: results of the MOSCATO 01 trial. *Cancer Discov*. 2017;7(6):586-595.
68. Tourneau CL, Delord JP, Gonçalves A, et al. Molecularly targeted therapy based on tumour molecular profiling versus conventional therapy for advanced cancer (SHIVA): a multicentre, open-label, proof-of-concept, randomised, controlled phase 2 trial. *Lancet Oncol*. 2015;16(13):1324-1334.
69. Rodon J, Soria JC, Berger R, et al. Genomic and transcriptomic profiling expands precision cancer medicine: the WINTHER trial. *Nat Med*. 2019;25(5):751-758.
70. Trédan O, Wang Q, Pissaloux D, et al. Molecular screening program to select molecular-based recommended therapies for metastatic cancer patients: analysis from the ProfiLER trial. *Ann Oncol*. 2019;30(5):757-765.
71. Sweeney CJ, Hainsworth JD, Bose R, et al. MyPathway human epidermal growth factor receptor 2 basket study: pertuzumab + trastuzumab treatment of a tissue-agnostic cohort of patients with human epidermal growth factor receptor 2—altered advanced solid tumors. *J Clin Oncol*. 2024;42(3):258-265.
72. O'Dwyer PJ, Gray RJ, Flaherty KT, et al. The NCI-MATCH trial: lessons for precision oncology. *Nat Med*. 2023;29(6):1349-1357.

73. Sicklick JK, Kato S, Okamura R, et al. Molecular profiling of cancer patients enables personalized combination therapy: the I-PREDICT study. *Nat Med*. 2019;25(5):744-750.
74. Hoes LR, van Berge Henegouwen JM, van der Wijngaart H, et al. Patients with rare cancers in the Drug Rediscovery Protocol (DRUP) benefit from genomics-guided treatment. *Clin Cancer Res*. 2022;28(7):1402-1411.
75. Vasudev NS, Scelo G, Glennon KI, et al. Application of genomic sequencing to refine patient stratification for adjuvant therapy in renal cell carcinoma. *Clin Cancer Res*. 2023;29(7):1220-1231.
76. Punekar SR, Velcheti V, Neel BG, Wong KK. The current state of the art and future trends in RAS-targeted cancer therapies. *Nat Rev Clin Oncol*. 2022;19(10):637-655.
77. Dumbrava EE, Johnson ML, Tolcher AW, et al. First-in-human study of PC14586, a small molecule structural corrector of Y220C mutant p53, in patients with advanced solid tumors harboring a TP53 Y220C mutation. *J Clin Oncol*. 2022;40(suppl 16):3003.
78. Gerlinger M, Rowan AJ, Horswell S, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. *N Engl J Med*. 2012;366(10):883-892.
79. Turajlic S, Xu H, Litchfield K, et al. Tracking cancer evolution reveals constrained routes to metastases: TRACERx Renal. *Cell*. 2018;173(3):581-594.e12.
80. Turajlic S, Xu H, Litchfield K, et al. Deterministic evolutionary trajectories influence primary tumor growth: TRACERx Renal. *Cell*. 2018;173(3):595-610.e11.